

Prevention of Initial Attacks of Rheumatic Fever

MILTON S. SASLAW, M.D., JAMES M. JABLON, Ph.D., and JOHN A. MAZZARELLA, M.D.

THE DEVELOPMENT of effective programs of preventive medicine depends largely on improvements in public health methodology. The epidemiologic approach which attempts to evaluate these methods refers not only to the review and analysis of known disease occurrence and distribution but also to the orderly method of gaining new knowledge.

Based on these concepts, the department of medical research of the National Children's Cardiac Hospital, with the cooperation of the Florida State and Dade County Health Departments and the Dade County Board of Public Instruction, and with the support of the Heart Disease Control Program of the Public Health Service, undertook the evaluation of an approach to the prevention of initial attacks of rheumatic fever. The occurrence of rheumatic fever is predicated on the epidemiology of group A beta hemolytic streptococci, and prevention of initial attacks depends upon early and complete treatment of group A streptococcal infections (1). Collection of data relative to incidence and prevalence and rapid identification of these organisms in the Miami, Fla., area

was deemed essential. General bacteriological techniques, the standard procedures, are often too time consuming, and the positive identification of the streptococci may be delayed for several days. This may be critical for effective treatment.

Since the development of the fluorescent antibody (FA) technique by Coons and associates (2), in a study on localization of antigen in tissue cells, the technique has been used to identify many bacteria (3-8), viruses (9-12), fungi (13), and protozoan parasites (14-15). The rapid identification of group A streptococci, in pure cultures as well as in throat swabs, was explored by Moody and co-workers (16). By this method group A organisms could be identified in a few hours rather than days.

In the present program for prophylaxis, the FA technique was investigated as a means of rapidly identifying group A streptococci in throat cultures.

Methods

Each cooperating agency reviewed and approved the protocol prior to initiation of the study. The director of the division of research for business and industry of the University of Miami stratified the white elementary schools of Dade County into high, middle, and low socioeconomic classes, based on the official 1960 census tracts. Negro schools of mixed socioeconomic strata were listed separately.

The list of schools was then submitted to a statistician of the Heart Disease Control Program of the Public Health Service, who provided a statistical sampling of these schools.

Dr. Saslaw is director of medical research and Dr. Jablon is microbiologist, National Children's Cardiac Hospital, Miami, Fla. Dr. Saslaw is also associate professor of preventive medicine, University of Miami School of Medicine, Coral Gables, Fla. Dr. Mazzarella, a medical officer of the Public Health Service, at the time of the study was assigned to the Dade County Health Department. The study was supported by the Public Health Service through NIH grant H-4467 and heart disease control contract No. SAPH 76318.

Twelve schools, three each of the high, middle, and low socioeconomic white schools and three Negro schools, were chosen randomly. Each school was visited and the principal's approval of the study was received.

Using the tables of Fischer and Yates (17), a list of random numbers was compiled for each of the six grades in each school. Based on these numbers, 15 students were selected from each of these grades, a total of 1,080 children. Consent slips were distributed to the parents of these children, and 604 permissions (55.93 percent) were obtained. Table 1 lists the participating students by sex, socioeconomic level, and race. Our goal of five or more students per grade was met in all but one grade. Routine studies on this group of 604 students began October 3, 1960.

Beginning November 9, 1960, 4 of the 12 schools, 1 representing high, 1 middle, and 1 low socioeconomic white populations, and 1 mixed socioeconomic Negro group were chosen randomly for participation in an absentee program. Since the laboratory was able to make additional studies, four more schools, representing the same population categories, joined this portion of the project on January 9, 1961, 4½ months before its termination on May 25, 1961. Four schools were studied for 125 days, three

for 85, and one for 83, a total of 838 school days (table 2). A total of 394 children participated for 41,242 student days.

For the absentee portion of the program, each school morning, as promptly as possible, a list of the absent participating children was obtained. Under direct supervision of a physician, the cause of each absence was ascertained for as many children as could be reached by telephone. The names and addresses of children absent because of respiratory infection or fever of unknown origin, as well as the names and addresses of all absentee participants who could not be reached by telephone, were submitted to the supervisor of nurses at the Dade County Health Department.

For the routine portion of the program, each Monday two schools were visited. Each Tuesday one school was visited. The visits were scheduled so that no two schools of the same socioeconomic level were visited during the same week. Throat swabs were taken monthly on each participating student for 8 consecutive months, October through May. In October, January, and May venous blood samples for the determination of antistreptolysin O (ASO) titers were drawn from each participant by a physician or by an experienced technician under the supervision of the physician.

Throat swabs collected during the routine program were studied by the FA technique (16). Positive or equivocal specimens were also studied by the conventional bacteriological technique (18). At various times random samples were examined by both techniques to maintain continuous surveillance of the reliability of our methods.

County health nurses made house calls to 499 absentees, or 30 percent (table 2), to collect essential medical data and throat cultures and urine samples as indicated. Throat cultures were collected from 259 absentees, indicating that approximately half of the home visits were necessitated not by respiratory illnesses but by lack of telephones and consequent inability to communicate with the family except by direct visit. No special blood samples were drawn from this group of students.

Specimens were delivered to the department of medical research of the National Children's Cardiac Hospital usually by 1 p.m. Urine

Table 1. Students participating in rheumatic fever study, by race, socioeconomic level, school, and sex, Dade County, Fla., October 1960-May 1961

Race, socioeconomic level, and school	Male	Female	Total
White-----	219	199	418
High-----	56	66	122
C-----	19	20	39
K-----	22	22	44
CW-----	15	24	39
Middle-----	63	63	126
F-----	15	21	36
MS-----	25	19	44
SL-----	23	23	46
Low-----	100	70	170
EP-----	38	25	63
MC-----	30	27	57
HF-----	32	18	50
Negro-----	85	101	186
D-----	27	38	65
LC-----	25	33	58
RP-----	33	30	63
Total-----	304	300	604

Table 2. Summary of data on school absentees from eight elementary schools, Miami, Fla., October 1960–May 1961

Race, socio-economic level, and school	Enrollment		Number school days	Number student days	Absentees		Home visits			Cultures					
	In school	In project			Number	Percent of student days	Number	Percent of—		Number	Percent from absentees	Beta hemolytic streptococci recovered			
								Student days	Absentees			Total		Group A	
												Number	Percent	Number	Percent
White-----	3, 795	271	628	28, 467	1, 165	4. 1	302	1. 1	25. 9	201	17. 3	29	14. 4	17	8. 5
High-----	1, 452	78	210	8, 190	303	3. 7	62	. 8	20. 5	58	19. 1	11	19. 0	8	13. 8
C-----	774	39	85	3, 315	155	4. 7	21	. 6	13. 5	21	13. 5	5	23. 8	5	23. 8
CW-----	678	39	125	4, 875	148	3. 0	41	. 8	27. 7	37	25. 0	6	16. 2	3	8. 1
Middle-----	1, 287	80	208	8, 152	316	3. 9	80	1. 0	25. 3	63	19. 9	8	12. 7	6	9. 5
MS-----	563	44	83	3, 652	100	2. 7	23	. 6	23. 0	17	17. 0	0	0	0	0
F-----	724	36	125	4, 500	216	4. 8	57	1. 3	26. 4	46	21. 3	8	17. 4	6	13. 0
Low-----	1, 056	113	210	12, 125	546	4. 5	160	1. 3	29. 3	80	14. 7	10	12. 5	3	3. 8
EP-----	538	63	125	7, 875	401	5. 1	140	1. 8	34. 9	61	15. 2	5	8. 2	2	3. 3
HF-----	518	50	85	4, 250	145	3. 4	20	. 5	13. 8	19	13. 1	5	26. 3	1	5. 3
Negro-----	2, 664	123	210	12, 775	488	3. 8	197	1. 5	40. 4	58	11. 9	7	12. 1	4	6. 9
D-----	1, 497	65	85	5, 525	136	2. 5	75	1. 4	55. 1	14	10. 3	1	7. 1	1	7. 1
LC-----	1, 167	58	125	7, 250	352	4. 9	122	1. 7	34. 7	44	12. 5	6	13. 6	3	6. 8
Total-----	6, 459	394	838	41, 242	1, 653	4. 0	499	1. 2	30. 2	259	15. 7	36	13. 9	21	8. 1

specimens were examined for albuminuria and microscopic hematuria. All urinalyses were negative except for one specimen, which showed microscopic hematuria, which cleared up after 3 days.

Throat swabs were placed in Todd-Hewitt broth and incubated at 35°–37° C. for 3 hours. Each culture was studied by FA and conventional bacteriological techniques. Each child whose throat culture harbored group A streptococci had a repeat culture taken 10 to 14 days after the initial culture, and again 2 weeks later.

Bacteriological procedures. Fluorescein-conjugated group A streptococcal antiserum was supplied by the Communicable Disease Center, Public Health Service, Atlanta, Ga. Nonconjugated streptococcal antisera of groups A, B, C, D, F, and G streptococci were purchased commercially (A). The nonconjugated sera were used for the identification of streptococci by the conventional bacteriological method (18), using the precipitin technique of Lancefield (19).

Neopeptone and blood agar base plates con-

taining 4 percent defibrinated sheep blood were purchased commercially (B) and used as bacteriological media throughout the study. Todd-Hewitt broth (Difco) was used for growing the specimen throat cultures and for preparing extracts of the streptococci when the organism was isolated.

A Leitz Ortholux microscope equipped with a dark-field condenser, D=1.2A, a 10X periplan, wide-field ocular lens, and a 100X achromatic oil immersion objective equipped with a drop-in funnel to reduce the numerical aperture to 1.2, was used. The light source was an HBO-200 mercury vapor bulb (Osram) in a specially designed Leitz housing. The light filter system was a combination of a BG 12 barrier filter and an OG 1 ocular filter.

Regular 3" x 1" slides and 24 mm. square No. 1 Corning coverslips were used. One of the difficulties of the FA technique lies in the preparation of the specimen slides. During the process of staining with fluorescein-conjugated antiserum, considerable material, sometimes almost the entire specimen, may be

washed off the slide. Aside from the annoyance and loss of time, the specimen may be reported erroneously as negative. Toward the end of the present study this difficulty was overcome by preparing the slides in the following manner:

- Slides were cleansed thoroughly in 95 percent ethanol or methanol and dried.
- A solution of 0.75 percent gelatin in distilled water was prepared. Phenol, 0.5 percent, was added as a preservative.
- The cleaned slides were immersed in the gelatin solution for a few seconds.
- The slides were removed and placed upright on a piece of paper toweling to permit them to drain and dry.

The slides were coated with an even, thin film of gelatin and were stored in a slide box until ready for use. The thin film of gelatin does not interfere with fluorescence microscopy and it provides enough of an adhesive surface to facilitate the adherence of the specimen to the slide. The method is similar to that used by Coons and associates (2) in the preparation of tissue slides for fluorescence microscopy.

A solution of buffered glycerol-saline containing 90 percent glycerol and 10 percent buffered saline, pH 7.2, as recommended by Moody and associates (16), was used for mounting

coverslips on specimen slides. Nonfluorescing immersion oil and 0.01 M phosphate buffered saline were used.

The preparation of specimens for fluorescent microscopic study is that described by Moody and associates (16). A loopful of bacterial sediment, after 3 hours' growth in Todd-Hewitt broth, was used. Another loopful was used to streak a blood agar base plate. The plate was incubated overnight; isolated hemolytic colonies were fished to Todd-Hewitt broth; after overnight incubation, extracts of the broths were prepared by the Lancefield acid technique (19) or the Rantz and Randall (20) method. The extracts were then tested for the appropriate group by the capillary precipitin technique of Swift, Wilson, and Lancefield (21).

Results

Streptococcal recovery. Of 4,438 throat cultures from asymptomatic children, 4,046 were negative, 134 equivocal, and 258 positive by fluorescence. Equivocal and positive specimens, plus a randomly selected group of 176 negative specimens, a total of 568, were then examined by conventional bacteriological techniques. Group A beta hemolytic streptococci were pres-

Table 3. Results of examination of throat cultures from 4,438 asymptomatic school children by fluorescent antibody technique and identification of group A beta hemolytic streptococci by conventional bacteriological technique, by race and socioeconomic level, Dade County, Fla., October 1960-May 1961

Race and socioeconomic level	Number throat cultures	Fluorescent antibody technique ¹						
		Positive		Equivocal		Negative		
		Conventional bacteriological technique ²						
		Positive	Negative	Positive	Negative	Positive	Negative	Not done
White.....	3, 049	52	138	9	68	2	115	2, 665
High.....	859	7	45	4	21	0	43	739
Middle.....	959	33	42	1	25	2	52	804
Low.....	1, 231	12	51	4	22	0	20	1, 122
Negro.....	1, 389	28	40	13	44	1	58	1, 205
Total.....	4, 438	80	178	22	112	3	173	3, 870

¹ Beta hemolytic streptococci.

² Group A only.

ent in 105 (18.5 percent), or 2.4 percent of all cultures taken (table 3). The FA technique identified group A beta hemolytic streptococci in 258, or 5.8 percent. The combined techniques identified group A streptococci in 283 samples (6.4 percent).

Agreement between the FA findings after 3 hours of incubation of the throat swabs and results by conventional bacteriological techniques was more than 90 percent for both absentees and nonabsentees. The number of positive group A cultures recognized by the FA technique was always greater than the number found by the conventional method (table 4). The positive cultures among the nonabsentees, children apparently in good health, might indicate a carrier state, especially where the number of group A streptococci in the throat was small.

Correlation between results with the FA technique and with conventional methods is heavily weighted when throat swabs are negative. This indicates that the FA technique is an excellent screening procedure; if a culture is negative by the FA method it is probably negative by conventional cultural methods. Only 16 (2.5 percent) throat swabs negative by the FA technique were positive by conventional methods.

Evaluation of positive results depends on whether the conventional bacteriological

method, the FA technique, or a combination of these is considered as standard. In this study, if the bacteriological procedure is accepted as the standard, only 50 throat cultures would have been identified as positive for group A streptococci; 34 (68 percent) of these were also found by the FA technique, while 16 (32 percent) were missed. If the FA technique is accepted, 83 cultures would have been considered positive; 34 (41 percent) of these were also identified by bacteriological methods and 49 (59 percent) were missed. Many of these 49 cultures, if studied intensively, undoubtedly would have been shown to contain group A streptococci. Such intensive study was not done, however; only two throat swabs positive by FA technique and originally nonbeta hemolytic were followed intensively. Both proved to be group A organisms.

In a similar study in Chicago (22), a considerable number of FA positive, bacteriologically negative cultures were found positive after intensive bacteriological investigation, and agreement between results with FA and conventional bacteriological methods was increased from 72 to 94.6 percent.

Since each technique, FA and conventional, shows a substantial number of unrecognized group A streptococci, combination of the two methods gave the most practical results; on this

Table 4. Agreement between results of examination of 735 throat swabs for group A beta hemolytic streptococci taken from 604 children in 12 elementary schools, Miami, Fla., 1959-61

Culture (bacteriological)	Fluorescent antibody technique								
	Absentees ¹			Nonabsentees ²			Total ³		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive.....	10	4 8	18	24	8	32	34	16	50
Negative.....	10	223	233	39	413	452	49	636	685
Total.....	20	231	251	63	421	484	83	652	6 735

¹ 92.8 percent agreement, 7.2 percent disagreement.

² 90.3 percent agreement, 9.7 percent disagreement.

³ 91.2 percent agreement, 8.8 percent disagreement.

⁴ Not tested by FA technique because of mechanical breakdown of equipment.

⁵ Includes 568 cultures, 10/60-5/61, less 134 equivocal cultures, plus 301 cultures studied 1/60-5/60 in a pilot investigation.

NOTE: Agreement, $\frac{(FA+, culture+) + (FA-, culture-)}{\text{Total swabs}}$

Disagreement, $\frac{(FA+, culture-) + (FA-, culture+)}{\text{Total swabs}}$

basis 99 cultures were positive (table 4). By the FA technique, 83 (83.8 percent) group A streptococci were recognized, while by conventional bacteriological methods only 50 (50.5 percent) were isolated.

In the identification of group A streptococci some difficulties may be encountered with the FA technique. Staphylococci, for example, often show a high degree of nonspecific fluorescence with group A fluorescein-conjugated antiserum. However, with increased experience, staphylococci can be readily differentiated from group A streptococci.

A more important problem is the cross reactivity sometimes found with streptococci other than group A, usually group C or group G strains. Redys and associates (23) reported good results in inhibiting the reactivity of these

organisms by adding nonconjugated group C antiserum to test cultures prior to staining with fluorescein-conjugated group A antiserum.

A third source of difficulty arises with some streptococci which show fluorescence with group A fluorescein-conjugated antiserum but either fail to grow out, perhaps because of nonviability of the organisms, or remain unrecognized because of failure to develop as beta hemolytic colonies by conventional methods. This problem is under further investigation.

Based on results of conventional procedures, Negro students have a higher streptococcal recovery rate than white students, but results with the fluorescent and combined methods contradict this (table 5). White students from the high and the low socioeconomic areas show streptococcal recovery rates comparable

Table 5. Throat cultures from school children positive for beta hemolytic streptococci by conventional bacteriological and fluorescent antibody techniques, by race and socioeconomic level, Dade County, Fla., October 1960–May 1961

Race and socioeconomic level	Number throat cultures	Conventional bacteriological technique				Fluorescent antibody technique (group A only)		Both methods (group A only)	
		All beta streptococci		Group A		Number	Percent	Number	Percent
		Number	Percent	Number	Percent				
White.....	3, 049	82	2. 7	63	2. 1	190	6. 2	201	6. 6
High.....	859	15	1. 7	11	1. 3	52	6. 1	56	6. 5
Middle.....	959	45	4. 7	36	3. 8	75	7. 8	78	8. 1
Low.....	1, 231	22	1. 8	16	1. 3	63	5. 1	67	5. 4
Negro.....	1, 389	62	4. 5	42	3. 0	68	4. 9	82	5. 9
Total.....	4, 438	144	3. 2	105	2. 4	258	5. 8	283	6. 4

Table 6. Throat cultures from school children positive for group A beta hemolytic streptococci by conventional bacteriological and fluorescent antibody techniques, by sex, Dade County, Fla., October 1960–May 1961

Sex	Number throat cultures	Positive by—					
		Conventional bacteriological technique		Fluorescent antibody technique		Both methods	
		Number	Percent	Number	Percent	Number	Percent
Male.....	2, 242	59	2. 6	137	6. 1	150	6. 7
Female.....	2, 196	46	2. 1	121	5. 5	133	6. 1
Total.....	4, 438	105	2. 4	258	5. 8	283	6. 4

Table 7. Results of examination of throat cultures from 4,438 asymptomatic school children, by month, Dade County, Fla., October 1960–May 1961

Month	Number throat cul- tures	Beta hemolytic streptococci										
		Conventional bacteriologi- cal technique				Fluorescent antibody technique						
		Total		Group A		Positive		Equivocal		Negative		
		Num- ber	Per- cent	Num- ber	Per- cent	Conventional bacteriological technique						
						Posi- tive	Nega- tive	Posi- tive	Nega- tive	Posi- tive	Nega- tive	Not done
1960												
October	585	30	5. 1	24	4. 1	17	43	7	24	0	47	447
November	585	18	3. 1	12	2. 1	4	8	8	52	0	2	511
December	579	24	4. 1	14	2. 4	7	10	5	31	2	89	435
1961												
January	557	21	3. 8	14	2. 5	12	17	2	3	0	2	521
February	552	18	3. 3	11	2. 0	11	20	0	2	0	0	519
March	532	22	4. 1	21	3. 9	20	24	0	0	1	9	478
April	527	10	1. 9	8	1. 5	8	22	0	0	0	20	477
May	521	1	. 2	1	. 2	1	34	0	0	0	4	482
Total	4, 438	144	3. 2	105	2. 4	80	178	22	112	3	173	3, 870

to those for Negroes; the rate is highest in white students from medium socioeconomic environments.

Males consistently had slightly higher recovery rates of beta hemolytic streptococci and group A beta hemolytic streptococci than females. This finding was independent of the technique used (table 6).

The majority of the questionable fluorescence findings (table 7) were encountered during the first 3 months of study. These correlated with the experience of a new technician.

During May 1961, 35 specimens were positive for group A streptococci by the fluorescent procedure (table 7). However, the conventional method confirmed this finding in only one case. Twenty-eight of these cultures were from students in only four classrooms.

The younger children had a slightly higher carrier rate than the children in grades 4 to 6. This finding was independent of the method of identification (table 8).

ASO titers. ASO titers were determined three times; the average titer was 131.9 Todd units. Students from whom no group A or-

ganisms were recovered had lower titers than those from whom group A streptococci were recovered at least once, regardless of whether illness occurred or not. The average titers of all participants are shown in table 9, by race, socioeconomic level, and school. Table 10 lists titers by school groups and by bacteriological findings.

Negro children had higher average ASO titers (170.1) than white children (114.6) (table 10). When group A organisms were recovered from the throats of Negroes, the titer level (160.4) was not above the average; the levels showed elevation in those from whose throats group C and group G strains (203.8 and 347.1) were isolated. Unfortunately, numbers of nonabsentee children with strains C and G were too small to compare with absentees to determine any possible correlation of clinical illness, presence of these organisms, and height of ASO titer.

ASO titer averages from white children whose throat cultures yielded group A isolates were consistently higher than overall ASO averages. When group A organisms were recov-

Table 8. Results of examination of throat cultures from 4,438 asymptomatic school children, by grade, Dade County, Fla., October 1960–May 1961

Grade	Number throat cultures	Beta hemolytic streptococci										
		Conventional bacteriological technique				Fluorescent antibody technique ¹						
		Total		Group A		Positive		Equivocal		Negative		
		Number	Percent	Number	Percent	Conventional bacteriological technique ¹						
						Positive	Negative	Positive	Negative	Positive	Negative	Not done
1	608	21	3.5	15	2.5	14	36	1	11	0	24	522
2	693	20	2.9	18	2.6	16	40	2	15	0	22	598
3	716	26	3.6	19	2.7	12	29	7	19	0	29	620
4	860	27	3.1	20	2.3	14	28	6	24	0	34	754
5	741	24	3.2	14	1.9	11	24	0	22	3	29	652
6	820	26	3.2	19	2.3	13	21	6	21	0	35	724
Total	4,438	144	3.2	105	2.4	80	178	22	112	3	173	3,870

¹ Group A only.

ered, ASO titer levels were lower when children were absent because of respiratory illnesses than when the children were not absent or were absent because of nonrespiratory illnesses. A fascinating observation is the almost consistently higher ASO levels among nonabsentees

compared with these levels among absentees. These findings raise the question of the streptococcal origin of some of the respiratory illnesses in this study.

Titers for students from whom group A organisms were recovered followed the same pattern of distribution as titers for students from whom group A streptococci were recovered by the FA technique. The ASO titers do not correlate with the recovery of group A organisms by the conventional technique alone.

Treatment. When physicians and parents were notified of the presence of group A beta hemolytic streptococci within 24 hours of onset of clinical symptoms, they agreed to recommended treatment. All 20 children ill with respiratory infections who were found to harbor group A streptococci by FA technique received treatment (table 11). Of 11 other children whose throats yielded strains proved to be group A by the conventional technique (during mechanical breakdown of fluorescence microscope), treatment was recommended for 9, and 5 children (55.6 percent) accepted and received therapy. Five additional children were found to carry group A streptococci, by conventional bacteriological methods, but identification was so long delayed that notification

Table 9. Antistreptolysin O titers among 581 school children participating in total program, Dade County, Fla., October 1960–May 1961

Race, socioeconomic level, and school	Average, all participants	No group A streptococci	Group A streptococci
White	107.8	97.4	165.9
High	90.7	82.7	147.0
C	128.6	119.4	172.6
CW	56.4	46.6	152.9
K	85.5	83.3	104.2
Middle	123.0	102.6	191.3
MS	149.3	113.2	523.7
F	117.8	94.3	152.5
SL	100.6	94.8	118.7
Low	108.2	102.2	139.9
EP	121.2	117.3	153.9
HF	101.9	92.0	144.0
MG	98.6	98.7	96.8
Negro	179.3	183.3	162.0
D	163.2	165.5	153.9
LC	177.7	180.6	166.9
RP	197.6	203.4	166.4
Total	129.6	122.8	164.5

of findings and recommendations for treatment were omitted. Only one child had streptococci in his throat 2 weeks after initial treatment. All followup cultures on the other 24 treated children were negative.

Discussion

The chief value of the FA technique lies in its use as a rapid and sensitive diagnostic procedure. Improvement in materials and techniques presages the usefulness of this procedure as a guide to early and effective treatment of group A streptococcal infections to prevent initial attacks of rheumatic fever.

The application of the FA technique in conjunction with routine bacteriological procedures increases the recovery rate of group A beta hemolytic streptococci considerably when compared with results of routine bacteriological procedures alone. Consequently, the FA technique permits appreciation of a truer pattern of distribution of group A beta hemolytic streptococci.

Socioeconomic level does not appear to play the same role in the streptococcal carrier rate in the Miami area as in other areas of the United States. Griffith has noted the role of poor nutrition, group crowding, and the associated streptococcal carrier rate in contributing to

Table 11. Treatment given, according to group A beta streptococci found, 36 elementary school children, Miami, Fla., October 1960–May 1961

Test results ¹	Positive cases found		
	Total	Treated	
		Num-ber	Per-cent
FA positive, C positive.....	10	10	100
FA questionable, C negative.....	² 5	0	² 0
FA questionable, C positive.....	³ 3	1	³ 33
FA positive, C negative.....	10	10	100
FA negative, C positive.....	⁴ 8	4	⁴ 50
Total.....	36	25	69

¹ FA, fluorescent antibody technique; C, conventional bacteriological technique.

² No treatment recommended.

³ No treatment recommended in 2 instances; fluorescence microscope broken and long culture delay.

⁴ Culture delay, mechanical breakdown of fluorescence microscope; 4 children asymptomatic and back in school at time of report.

susceptibility to rheumatic fever (24). Paul's studies in New Haven indicated that the incidence of rheumatic fever is eight times greater among children of the poorer classes (25). Clarke has shown that rheumatic fever appears to coincide closely with density of popu-

Table 10. Average antistreptolysin O titers for 1,033 blood samples taken from 303 elementary school children participating in absentee program, Miami, Fla., October 1960–May 1961

Streptococci group	Race and socioeconomic level					
	White				Negro	Total
	High	Middle	Low	Total		
Number specimens.....	202	219	290	711	322	1,033
Absentees.....	129	130	189	448	164	612
Nonabsentees.....	33	34	38	105	62	167
Average titer ¹	93.6	135.8	113.2	114.6	170.1	131.9
Absentees.....	85.1	101.4	102.1	97.0	165.0	115.2
Nonabsentees.....	71.1	122.6	122.1	106.2	158.1	125.5
Beta hemolytic streptococci, group—						
A.....	166.0	235.8	148.3	189.1	160.4	178.7
Absentees (A only).....	166.0	161.1	141.7	155.7	159.3	156.9
Nonabsentees (A only).....		568.2	187.2	415.8	163.0	281.8
B.....	6.0		101.0	66.5	180.3	90.9
C.....	100.0	166.0		126.2	203.8	187.0
G.....	6.0	6.0	141.1	112.7	347.1	188.0
Nongroupable.....	249.7	166.0		207.8		207.8

¹ Todd units.

lation, overcrowding of living quarters, and proximity to low-lying areas near rivers and waterways because all these factors favor the spread of upper respiratory infections (26). Curiously, these very factors are commonly found among the poorer classes in Miami, but these classes showed the lowest streptococcal recovery rate.

The higher recovery rate of group A organisms among male students fails to correlate with the fact that rheumatic fever occurs more frequently in females than in males (27).

Seasonal variation in recovery of group A organisms was demonstrated by the combined FA and conventional methods. The two periods of high recovery, one during October, shortly after school began, and another in March, may represent the transmission of strains of group A streptococci new to the environment of the recently convened classes, followed by relative herd immunity, with a subsequent gradual rise in streptococcal occurrence during early spring following the influx of tourists into the Miami area. This was not clearly demonstrated by the results with the conventional method alone. The fluorescent technique permitted the identification during May of a group of organisms which failed to show beta hemolysis.

Comparable to the results of previous studies conducted in the Miami area which showed a higher recovery rate of streptococci in children aged 6 to 9 years than in older populations (28), the recovery rates in this study varied inversely with the age (grade) of the students. This parallels the higher incidence rates of rheumatic fever found in the age range 6 to 9 years throughout other areas of the United States. Once again, the results were more distinct by the fluorescent and combined methods than with conventional procedures alone.

Recoveries of all groups of beta hemolytic streptococci and of group A organisms only were infrequent. Rates of infection with group A streptococci were lower in 1960-61 than during any of 9 previous years of study, 8.1 percent (table 2) against 14 percent (29-31). These rates were approximately the same in the various population groups except for those in the high socioeconomic level, which were higher than the other groups; the isolation rate for group A varied inversely with the economic

level. Recovery rates of total beta hemolytic and of group A streptococci showed no variation when related to sex, race, or school grade.

Although the absentee rate of 4.0 percent (table 2) was approximately the same as that found in previous studies, the group A streptococcal recovery rate of 8.1 percent was lower than the usual observation for the general school population (31), about 14 percent. No definite explanation can be offered for this finding, except perhaps that in the Miami area the streptococcal prevalence rate was low. The alternate possibility, that the FA technique might have failed to identify streptococci, is unlikely, since repeated bacteriological checks during the year revealed no failure of the FA procedure. Among absentees, the eight group A streptococci identified by the standard grouping and not by the FA method (table 12) occurred when the ultraviolet microscope suffered a light source failure.

Sociological Aspects

Despite the care exercised in selection and random sampling techniques, to provide adequate control and experimental data, certain problems and interesting unanticipated circumstances occurred. Five of these special sociological situations were considered worthy of reporting.

Selection of schools. Selection of schools according to census tract data can be mislead-

Table 12. Group A beta streptococci isolated from 259 throat cultures from absentee elementary school children, Miami, Fla., October 1960-May 1961

Fluorescent antibody technique	Conventional bacteriological technique		
	Posi- tive	Nega- tive	Total
Positive.....	10	10	20
Questionable.....	3	5	8
Negative.....	¹ 8	223	231
Total.....	21	238	259

¹ Not tested by fluorescent antibody technique because of mechanical breakdown of equipment.

Table 13. Number and percentage of students participating among schools in absentee program, Dade County, Fla., October 1960–May 1961

Race, socioeconomic level, and school	Number of students		Percent participation	
	Enrolled	In project	Total	Corrected ¹
White.....	3, 795	271	7. 1	50. 2
High.....	1, 452	78	5. 4	43. 3
C.....	774	39	5. 0	43. 3
CW.....	678	39	5. 8	43. 3
Middle.....	1, 287	80	6. 2	44. 4
MS.....	563	44	7. 8	48. 9
F.....	724	36	5. 0	40. 0
Low.....	1, 056	113	10. 7	62. 8
EP.....	538	63	11. 7	70. 0
HF.....	518	50	9. 7	55. 6
Negro.....	2, 664	123	4. 6	68. 3
D.....	1, 497	65	4. 3	72. 2
LC.....	1, 167	58	5. 0	64. 4
Total.....	6, 459	394	6. 1	54. 7

¹ Per school, based on 90 permission forms distributed in each school.

ing. One of the three schools originally selected in the white, low economic group was school E, in tract L. When a visit was made to this school to make arrangements to carry out the investigation, the number of children of professional parents was found to be high. An attempt to reconcile the census tract data with the high social level revealed that a railroad ran north and south close to the eastern boundary of the tract. School E was in the very narrow section between the eastern boundary and the railroad line, and served not tract L but tract H contiguous to it on the east, the highest income tract in Dade County. The majority of the population of tract L resided west of the railroad and were indeed a low-income group. They attended another school. Needless to say, a different school was substituted for school E, to represent a low-income population.

School response. Since the same number of students was canvassed in each grade in each school, regardless of the number enrolled, no statistical analysis is warranted. However, factors which seem to influence the percentage of students participating in the study include size of school, interest of the principal, and socioeconomic status of the children. As an exam-

ple, the percentage of Negroes participating in the study was the lowest of the socioeconomic groups (table 13), while the number of participating Negro students was the highest. Since 90 permission slips were distributed in each school, the low percentage of participation reflected the high total school enrollment of Negroes. The corrected percentages, based on the 90 permission forms, showed that the low-income white and total Negro groups responded more enthusiastically than the high- and middle-income children. School HF of the low-income group was intermediate in behavior, between low- and middle-income levels.

In all schools, the principals were cooperative and interested but occasionally were reluctant to disturb the parents, since the high- and middle-income groups generally used the services of private physicians and manifested an intolerance to research—"I don't want my child to be treated as a guinea pig."

Home visits. Since primary communication was by telephone, the frequency of "communication" visits was inversely proportional to the number of telephones in an area. Visits were relatively infrequent among high- and middle-income groups, more visits were required by low-income white populations, and the rate was very high among the Negroes (table 2). The difference between the number of home visits and the number of throat cultures taken suggests that telephone density in relation to population density may serve as an index of economic, if not socioeconomic, status.

School HF in the low-income group did not follow the pattern expected; cultures were taken from 95 percent of the children visited. This discrepancy may indicate that the categorization of this school may be in error. School response and absentee data also suggest that it may be a mistake to classify income level according to census tract.

Group A streptococci. In routine studies, recovery rates of group A streptococci were higher in the middle-income white schools and in all Negro schools than in schools considered to represent high- and low-income white populations (table 14). Results of studies of absentees contrasted with results of routine studies showed an inverse relationship between streptococcal recovery rate and economic level. In

school HF of the low-income group, the percentage of total beta hemolytic streptococci recovered among absentees was similar to the percentage in the high-income population. Data on the Negro population were in accordance with what might have been expected of a mixed-income white group.

Antistreptolysin O. Antistreptolysin O data revealed no significant correlation with socioeconomic stratum, with absence from the school, or with absence due to respiratory illness. No significant difference in ASO titers among the white children of various socioeconomic levels could be correlated with average titers (table 9), but Negro children had higher average ASO levels than white children (table 10), a finding consistently observed in Miami (29). The ASO titer level for Negro children was unchanged when group A organisms were recovered but was elevated in children from whom strains of group C or group G beta hemolytic streptococci were isolated. Among white children, ASO titers were higher when strains of group A were found; ASO titer elevations were not likely to

be seen when strains of group C and G were isolated.

An unexpected finding was the higher average ASO level in each socioeconomic group (except the high) among children who were not absent at all during the school year than among those who were absent at least once. There was no important difference in titer levels among absentees whether the absences were due to respiratory or nonrespiratory causes.

Acceptance of Treatment

Examination by conventional bacteriological methods often resulted in delay in reporting positive observations until the child had recovered and returned to school. Under these conditions many parents hesitated to take the child to a physician and physicians often advised against treatment. None of this reluctance was observed when the FA technique was used, because it permits early identification of group A streptococci and notification of the parents and physicians within 24 hours, while the child is still clinically ill. All followup cultures were negative, except for one 2-week culture which yielded group A streptococci.

The success in acceptance of treatment in this study by parents and physicians when streptococcal presence was reported early may be compared with two other situations. In the first instance, reporting of diagnoses was delayed because the fluorescence microscope was out of order for several days. As a result, only 5 (45 percent) of 11 children harboring group A streptococci received adequate treatment.

A study in 1958-59 used only conventional bacteriological techniques (32). Followup data were collected, whenever possible, from attending physicians. Adequate therapeutic information was available on 65 of 115 illnesses. Beta hemolytic streptococci were eradicated in 24 of the 38 treated cases, and in 3 additional cases repeat cultures were positive for streptococci but not for group A. In 11 illnesses therapy failed to eradicate the group A strains. Thus, only 11 (33 percent) of 33 ill children harboring group A beta hemolytic streptococci were known to have received adequate treatment, when studied conventionally.

The 1960-61 investigation reported here has

Table 14. Group A streptococci recovered (routine and absentees) among schools participating in total program, Dade County, Fla., October 1960-May 1961

Race, socioeconomic level, and school	Percent beta hemolytic streptococci recovered			
	Routine examination		Examination of absentees	
	Total beta	Group A	Total beta	Group A
White-----	2.7	2.1	14.4	8.5
High-----	1.7	1.3	19.0	13.8
C-----	2.7	1.8	23.8	23.8
CW-----	1.3	1.3	16.2	8.1
K-----	1.1	.7		
Middle-----	4.1	3.9	12.7	9.5
MS-----	1.4	1.4	0	0
F-----	6.1	4.3	17.4	13.0
SL-----	7.1	5.9		
Low-----	1.8	1.3	12.5	3.8
EP-----	1.5	.9	8.2	3.3
HF-----	1.5	1.0	26.3	5.0
MG-----	2.3	2.2		
Negro-----	4.5	3.0	12.1	6.9
D-----	4.2	3.1	7.1	7.1
LC-----	5.5	3.2	13.6	6.8
RP-----	3.8	2.7		
Total-----	3.2	2.4	13.9	8.1

demonstrated the possibility of providing adequate treatment to children clinically presenting respiratory or febrile symptoms sufficient to occasion absence from school, whose throat cultures reveal the presence of group A streptococci by FA technique. However, the present study design reaches only overt infections; it cannot be expected to prevent all cases of rheumatic fever, since rheumatic fever may follow occult streptococcal infections.

Results of the study indicate that sex, race, and socioeconomic status are not the major factors in the pathogenesis of rheumatic fever, at least not in the Miami area. Such factors as climate, heredity, metabolic anomalies, and hypersensitivity remain to be further evaluated. The specific relationship of the group A streptococcus to the host demands further scrutiny. Other factors not as yet defined but operating concordantly with the streptococci must be sought. Clearly, epidemiologic studies, using similar methodology, must be conducted simultaneously in areas of varying rheumatic fever incidence if data are to be validly comparable on a national level.

Summary

In Dade County, Fla., the fluorescent antibody (FA) technique was investigated as a means of rapidly identifying group A streptococci in throat cultures of a random sample of school children in the elementary grades in 12 schools. Absentees with respiratory infections as well as children attending classes were included in the study. Both the FA technique and conventional bacteriological methods were used in studying 735 throat swabs from 604 children.

The correlation of results by both methods was good: 91 percent of the overall findings, both positive and negative, were in agreement. Only 2.4 percent of the swabs positive by the conventional method were not recognized by the FA technique. The FA technique also indicated more group A streptococci than did routine bacteriological techniques, and special emphasis is placed on its relationship to early diagnosis and effective treatment of group A streptococcal infections.

Twenty children were clinically ill and ab-

sent from school because of respiratory or febrile symptoms. Group A beta hemolytic streptococci were promptly identified with the FA technique, and all 20 children were treated. When reporting of bacterial findings depended on conventional cultural methods, only 45 percent of the ill children received treatment.

Among white absentee children the recovery rates of group A beta hemolytic streptococci were directly proportional to income level; among Negroes rates were between those for low- and middle-income whites. The greatest difficulty in obtaining cooperation with the study was encountered among whites in the high socioeconomic level; Negroes cooperate well with a medical research program.

The data revealed no significant correlation between ASO titers and socioeconomic strata or absence from school due to respiratory illness or other causes. The average titer for Negroes was higher than for any white group. ASO averages did not correlate with white income levels. When associated with recovery of group A beta hemolytic streptococci the average ASO titers were inversely proportional to income level among whites; there was no rise in ASO titer among Negroes.

Selection of schools as representative of specific socioeconomic levels can be based on income of residents within a census tract, but only after inspection of other factors which might operate contrary to expectation. Concentration of telephones in relation to concentration of population is a valuable aid in classification of socioeconomic strata.

The project demonstrates that patients harboring group A beta hemolytic streptococci during clinical illness can be adequately treated, as an approach to the prevention of original episodes of rheumatic fever. The study also demonstrates considerable inconsistency in the incidence and prevalence of streptococci as they relate to rheumatic fever in Dade County, Fla., and elsewhere in the United States. The inconsistencies appear to correlate with relative infrequency of rheumatic fever in the Miami area.

Simultaneous epidemiologic studies are needed in geographic areas having a varying incidence of rheumatic fever, using similar methodology to permit valid comparisons.

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New Antimalarial Drug

In a clinical trial of an experimental antimalarial drug, CI501, a single injection has continued to protect volunteers from malaria after almost a year. Bites of heavily infected mosquitoes did not cause malaria in these subjects, while control subjects invariably developed the disease when bitten by the same mosquitoes.

The clinical trials of the drug were conducted by Dr. G. Robert Coatney, Dr. Peter G. Contacos, Dr. Harvey A. Elder, and John W. Kilpatrick of the National Institutes of Health, Public Health Service, and were reported at the November 1962 meeting of the American Society of Tropical Medicine and Hygiene. Laboratory and animal studies of the drug were reported by investigators from Parke, Davis and Company and the Christ Hospital Institute of Medical Research in Cincinnati, Ohio.

Dr. Coatney and his associates conducted the trials at the Federal penitentiary in Atlanta, Ga. Some 50 volunteers among the inmates participated. The first injections of the drug were given to five volunteers on November 24, 1961. The intramuscular injections were given at a dosage of 5 milligrams per kilogram of body weight. Within 2 months, 25 more volunteers received the drug. Most of the subjects were bitten by heavily infected mosquitoes, *Anopheles quadrimaculatus*, about a week after receiving the drug; a few were not challenged until 5½ months after the drug injections.

None of the volunteers given the drug have developed any evidence, clinical or parasitological, of malaria. Each received only one injection of the drug. Thus far the drug has been protective 10 times longer than conventional suppressives. No toxic side effects have been observed.

A slightly different form of the drug was tested

for its protective effect in a separate investigation. Of nine volunteers injected with this drug, one developed malaria 169 days after the first challenge. He had been challenged on the 6th day and again on the 79th day after receiving the drug.

The possible curative effect of CI501 was tested on malaria patients. Their symptoms disappeared and an apparent cure was effected. It is too soon, however, to definitely say that the drug is therapeutically effective, the investigators said. Quite possibly, the drug eliminates the parasite before infection takes root; studies of this point are underway. The scientists believe that, when the drug is injected, it is held in the intramuscular tissues, where it releases its effective materials into the bloodstream. Whether the parasites that typically form a reservoir in liver cells are actually killed is still undetermined.

CI501 is a pamoic acid salt of the base, 4,6-diamino-1-(p-chlorophenyl)-1, 2-dihydro-2, 2-dimethyls-triazine. Eleven years ago, this base was found to be formed in the body from the antimalarial drug, chlorguanide hydrochloride.

The investigators cautioned that, although CI501 has produced "spectacular" results in the preliminary trials, its effect under field conditions is still to be tested. If the promise of the clinical trials is borne out, the drug may greatly increase the chances of success in worldwide malaria eradication efforts. Each year, malaria still threatens more than a billion people, despite campaigns which, as of January 1960, had eradicated malaria from 18 countries and territories with a total population of 108 million. Malaria eradication efforts have reduced infant mortality 50 percent in British Guiana and have dropped the number of malaria patients in Greece from 1,000,000 to 1,200.